

ANALYSIS OF ISOZYME VARIATION IN NATURAL POPULATIONS OF JUNIPERUS ASHEI

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The study of isozymes is now a common part of scientific endeavor, but the recognition of multiple forms of enzymes within an organism is relatively recent, being announced in 1959 by Markert and Moller. Prior to that time, molecular heterogeneity noticed in enzyme preparations was attributed to contaminants or to denatured or degraded enzyme molecules (Markert, 1975). Two decades ago there were suggestions that this enzymatic heterogeneity might not be artifactual but might reflect reality within the cell (Neilands, 1952; Vesell & Bearn, 1957; Weiland & Pfeleiderer, 1957). Most of these early investigators used zone electrophoresis to separate enzyme preparations into components; however, these procedures were laborious and had poor resolving power. By coupling the starch gel electrophoresis techniques of Smithies (1955) with histochemical staining procedures to identify separate enzymes, Hunter and Market (1957) developed the zymogram technique, which had greater resolving power for the identification of the isozymes of many enzyme systems. This technique was first applied successfully to esterases and lactate dehydrogenases (Markert & Moller, 1959). The technique was direct, simple, easy to use, and applicable to enzymes in relatively crude homogenates. The development of the zymogram technique allowed recognition of isozymes as a natural and important aspect of cellular biochemistry that is found in nearly all organisms (Markert, 1975). Even greater resolving power was gained when acrylamide gel electrophoresis was introduced by Raymond and Weintraub (1959), and Ornstein and Davis (1959). As a result of the development of these techniques many investigators have focused on isozymes. The study of isozymes is important in order to understand a number of major biological problems, including the evolution of populations, the transformation of one gene into another, and the regulation of gene expression. Isozymes

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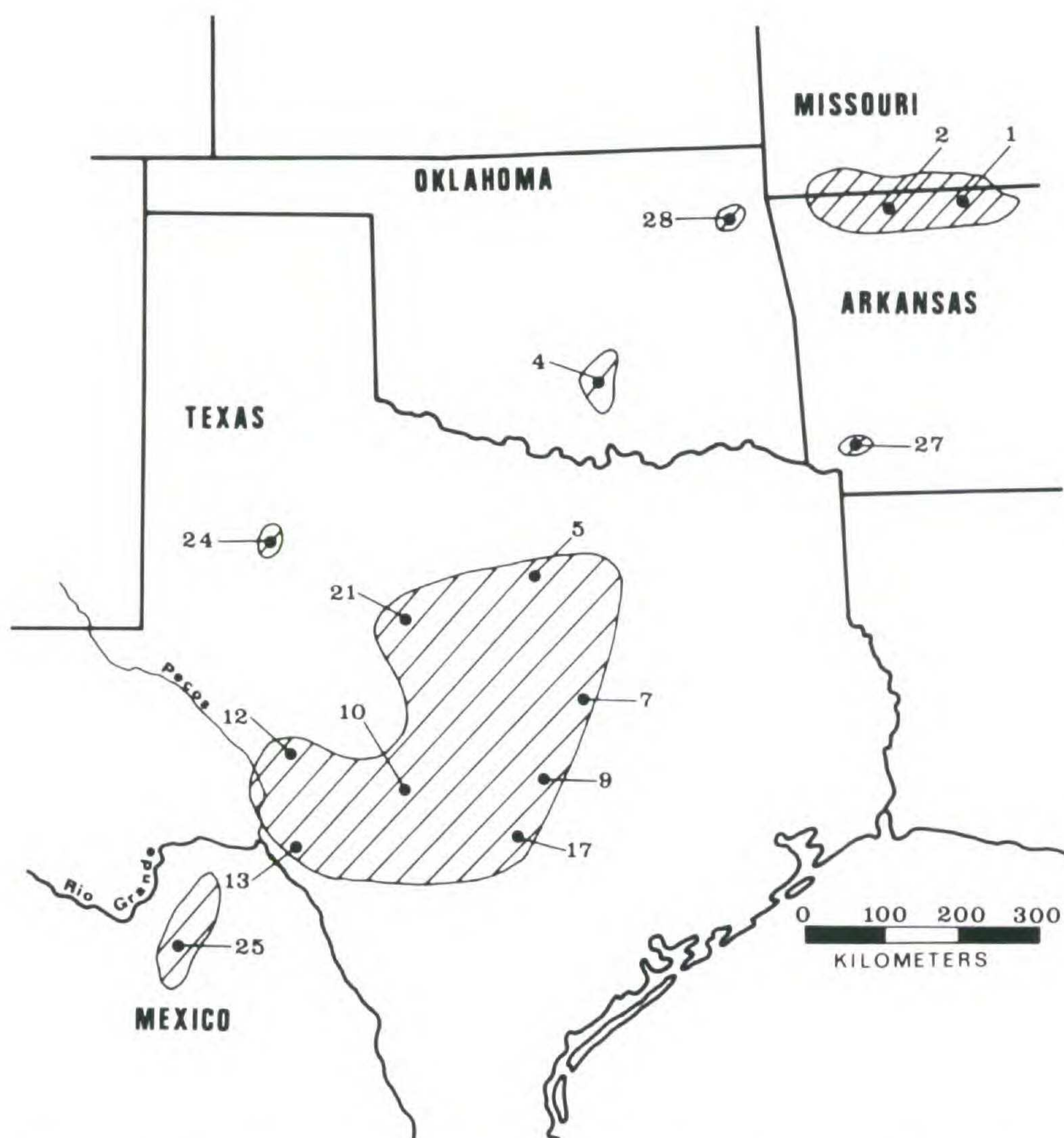


Figure 1. Distribution map of *Juniperus ashei* showing locations of population samples. Cross-hatched areas show approximate distributional ranges. Population numbers correspond to those used by Adams (1975, 1977).

may also hold the key to the understanding of metabolic regulation and the function of metabolic pathways in differentiated tissues (Horecker, 1975). These initial investigations of isozymes dealt primarily with animal systems. The plant biologists did not become intensely interested in isozymes until ten years ago (Shannon, 1968). At that time the idea that isozyme data might be useful in understanding evolutionary phenomena at the population level in plants was reviewed by Turner (1969) and Gottlieb (1971a).

The first investigations of isozymes in higher plants dealt predominantly with surveys of the various isoenzymatic systems found

in plant species (Shannon, 1968). Because of the widespread polymorphisms usually found in isozymes under natural conditions these data were normally more useful at the infraspecific levels than at higher categorical levels and seemed to be especially useful in studying evolutionary problems at the population level (Turner, 1969). There have been a few interesting and notable exceptions to this general principle. Isozymes have been used in interspecific studies on *Nicotiana* (Smith *et al.*, 1970; Sheen, 1970); *Datura* (Conklin & Smith, 1971); *Carthamus* (Efron *et al.*, 1972); *Lycopersicon* (Rich *et al.*, 1973); *Clarkia* (Gottlieb, 1973a, 1974a); *Stephanomeria* (Gottlieb, 1973b). These studies have been taxonomically oriented. On the other hand, infraspecific studies have been chiefly concerned with populational evolution, differentiation and variability. These kinds of studies include those on *Xanthium strumarium* (McMillan, 1975), *Stephanomeria exigua* spp. *coronaria* (Gottlieb, 1973c), *S. exigua* spp. *carotifera* (Gottlieb, 1974b), *Lupinus* and *Hymenopappus* (Babbel & Selander, 1975), *Betula populifolia* (Payne & Fairbrothers, 1973), *Avena barbata* and *A. fatua* (Allard *et al.*, 1972a; Allard & Kahler, 1973; Clegg & Allard, 1972, 1973; Hamrick & Allard, 1972; Marshall & Allard, 1969, 1970a, 1970b), *Bromus mollis* (Brown *et al.*, 1974), *Hordeum vulgare* (Allard *et al.*, 1972b; Clegg *et al.*, 1972; Kahler & Allard, 1970; Weir *et al.*, 1972, 1974), *Picea abies* (Tierstedt, 1973), *Pinus sylvestris* (Rudin, 1975; Rasmuson & Rudin, 1971; Rudin & Rasmuson 1973; Rudin *et al.*, 1974), *Pinus pungens* (Feret, 1974), *Pseudotsuga menziesii* (Muhs, 1974), *Cryptomeria japonica* (Sakai & Park, 1971), and *Thujaopsis dolabrata* (Sakai *et al.*, 1971).

The detection of hybridization with the use of isozymes has been investigated in *Phlox* (Levin, 1975) and *Typha* (Lee & Fairbrothers, 1973).

The inheritance of isozymes has been analyzed by many of the aforementioned workers as well as considerable research in the gymnosperm, forest trees such as *Picea abies* (Bartels, 1971; Lundkvist, 1974, 1975), *Pinus attenuate* (Conkle, 1971a,b), *Pinus sylvestris* (Rudin, 1975; Rudin & Rasmuson, 1973; Rudin *et al.*, 1974), and *Pinus nigra* (Bergmann, 1974). The general rule appears to be that most banding patterns are inherited co-dominantly (Allard *et al.*, 1975).

The literature on ontogenetic and seasonal variation has been reviewed by Kelley and Adams (1977) and the reader is referred

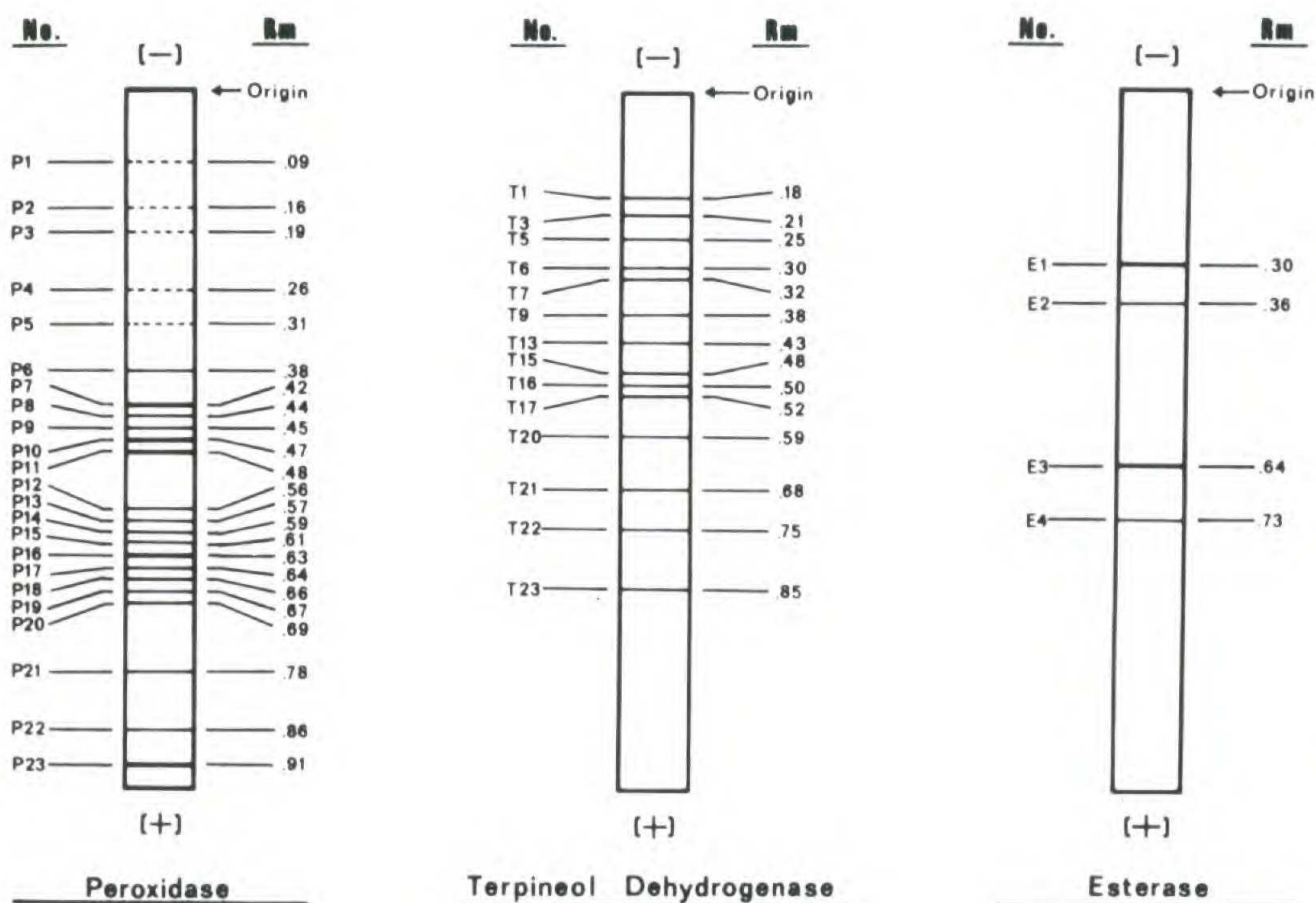


Figure 2. Summary of all possible isozymes observed in the *Juniperus ashei* trees examined. Rm = Relative Migration.

to that paper.

Several hypotheses have been proposed to explain the observed variations seen in the isozymes in these studies of various species. The isoallelic or neutral hypothesis suggests that much of the observed variation is physiologically irrelevant and the alleles are adaptively equivalent and their variation random (Kimura, 1968; Kimura & Crow, 1969; King & Jukes, 1969). In contrast, the selection hypothesis asserts that some form of natural selection is responsible for the maintenance of the variation (Gottlieb, 1971a; Powell, 1975). The disagreement between these two hypotheses is a significant matter, because according to the modern synthetic theory of evolution, natural selection is considered the basic force leading to genetic divergence and adaptation. The neutral hypothesis challenges this position by claiming that some variations at the molecular level are below the levels of selection.

The present isozyme study was undertaken to investigate the amount and nature of genetic variation in natural populations of *Juniperus ashei* Buch. (Cupressaceae). This species has been stud-

ied at the populational level by Adams and Turner (1970), and Adams (1975, 1977), using morphological and terpenoid data.

Juniperus ashei is an obligate outcrossing dioecious species that occurs on limestone outcrops and soils in central Texas, northern Mexico and in the Ozark Mountains of southern Missouri and northern Arkansas (Figure 1). Two small isolated populations of *J. ashei* are found in the northeastern portion of the species distribution: one in northeastern Oklahoma (23, Figure 1) and another in southwestern Arkansas (27, Figure 1). Both of these populations are in association with *J. virginiana*; *J. ashei* occupying the

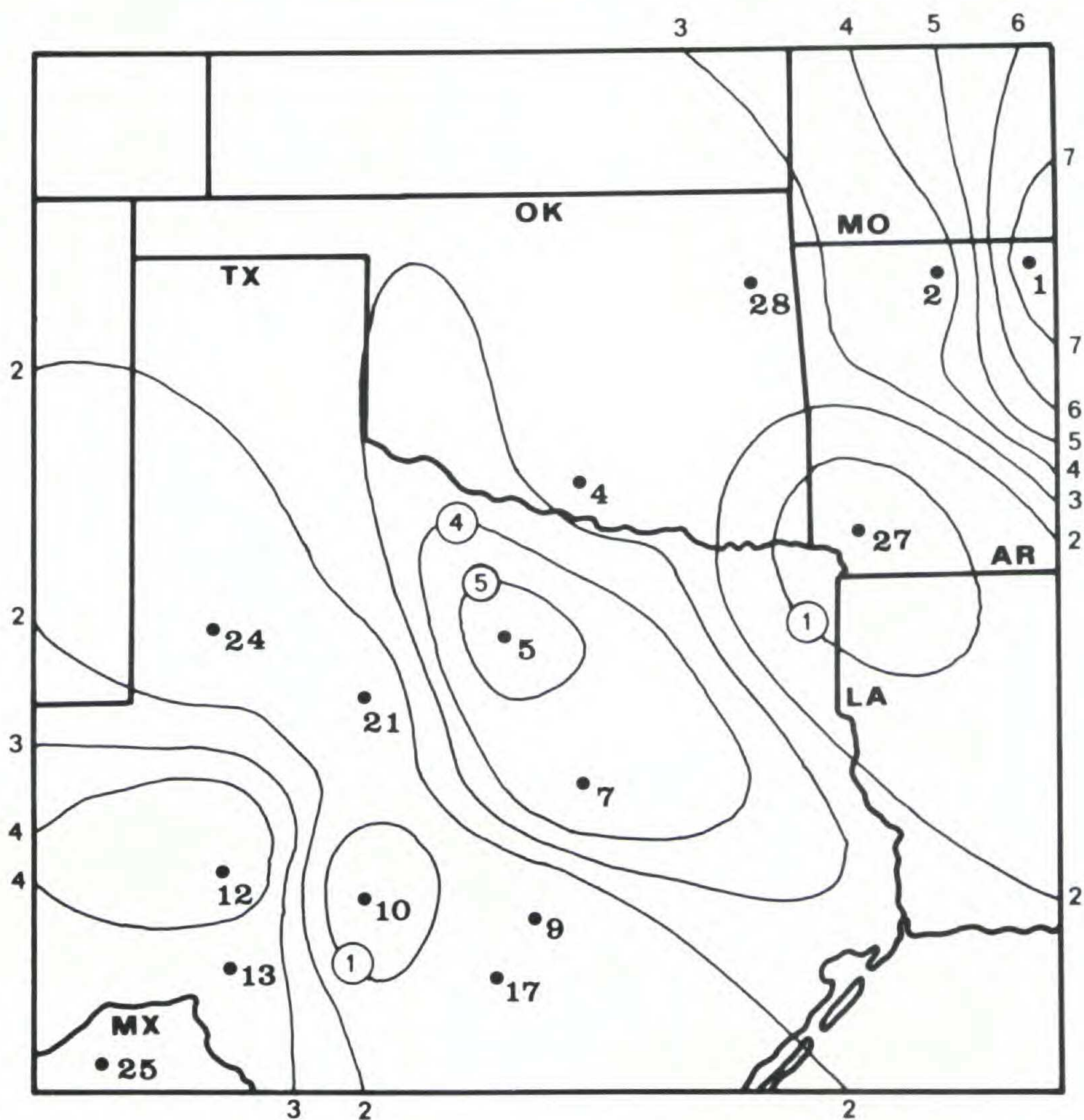


Figure 3. Contour map for average number of isoperoxidases per population of *Juniperus ashei* (Contours: 1 = 5.97; 7 = 10.13). Note the low number of bands at populations 27 and 10.

drier limestone soils at both sites. A small, pure stand of *J. ashei* is found in southcentral Oklahoma in the Arbuckle Mountains (4, Figure 1). The largest expanse of *J. ashei* is on the Edwards Plateau of central Texas. At the eastern edge of the Edwards Plateau, *J. ashei* is mixed with *J. virginiana* (7 & 9, Figure 1) and at the southwestern edge it occurs with *J. pinchotii* Sudw. (10, 12, 13, & 21, Figure 1). On the remaining portions of the Edwards Plateau *J. ashei* grows in relatively pure stands. A small isolated population of *J. ashei* is also found in west central Texas (24, Figure 1) in the more moist canyon floors with *J. pinchotii* occupying the drier sites. In the southwestern limits of its distribution *J. ashei* is found south of the Sierra del Carmen Mountains of Coahuila, Mexico with *J. pinchotii*, and occasionally with *J. flaccida* Schlect. (25, Figure 1).

Thus, by sampling throughout the natural range of *Juniperus ashei*, investigations can be conducted on the nature of isozyme variation in small isolated populations, in large continuous populations, and in populations at the center and the periphery of the species distribution.

MATERIALS AND METHODS

Sample Collection. Populations of *Juniperus ashei* were sampled from throughout its range. Figure 1 shows the locations of the populations sampled and the distribution of *J. ashei*. The population numbers shown in Figure 1 correspond to those used by Adams (1975, 1977) in his studies of this species (for exact locations, see Kelley, 1976). Whenever possible, 30 trees were sampled at random from each population. In populations that did not contain 30 trees, all trees present were sampled. The smallest number of trees sampled at any location was 19 (population 13 and 28).

Fresh foliage samples were collected in plastic bags during a two week period in November and December, 1974, and were stored at -7°C . within one hour from the time of collection. Samples were maintained at -7°C . until all samples were collected and returned to Colorado State University, Fort Collins, Colorado, where they were stored at -20°C . until the enzymes were extracted.

Enzyme Extraction. Populations were selected at random from cold storage and the enzymes of all samples from that population were extracted using the extraction buffer and procedures outlined

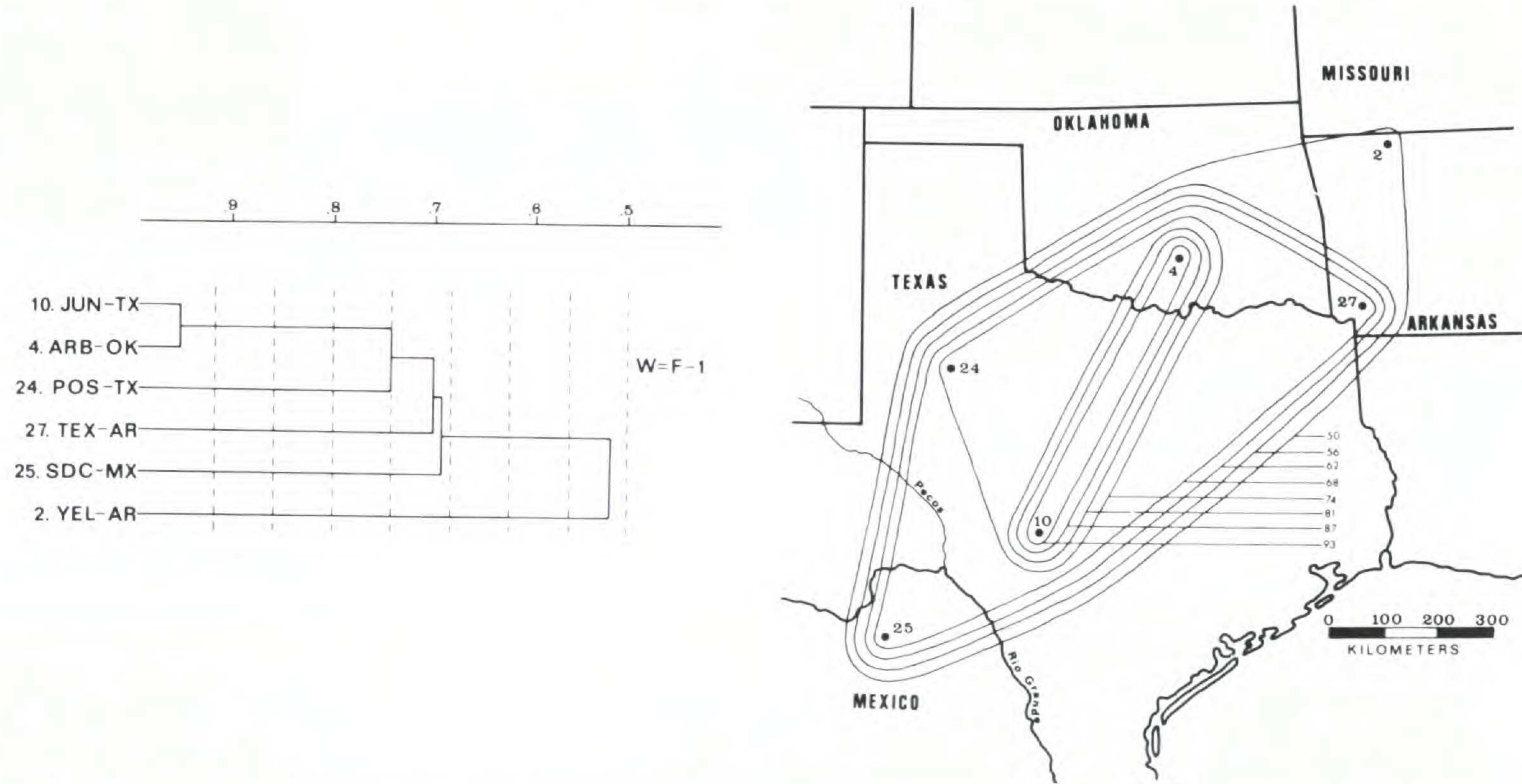


Figure 4. Phenogram and contoured similarity of six populations of *Juniperus ashei* based on four esterases, F-I weighted. Similarity values are shown on the scale at the top of the phenogram. The dashed lines on the phenogram show the contour levels used on the contoured similarity map.

by Kelley and Adams (1976). Enzyme extracts were stored at -20°C . until electrophoresis.

Samples were subjected to polyacrylamide gel electrophoresis within 96 hours from the time of extraction. Electrophoresis was performed as outlined by Kelley and Adams (1976), with $4\frac{1}{2}\%$, 6% , 8% anodic gels being used for peroxidases; 6% , 8% anodic gels being used for esterases and α -terpineol (alcohol) dehydrogenases.

Gels were stained for peroxidases, esterases, and α -terpineol (alcohol) dehydrogenases as outlined by Kelley and Adams (1976).

Following electrophoresis and staining, the Relative Migration (Rm) were calculated for all bands, data sheets were scored, and photographs were taken of the gels. DiCamelli and Bryan (1975) define Relative Migration as: $Rm = \text{migration of enzyme} / \text{migration of bromphenol blue}$. After all samples of *Juniperus ashei* were run the band numbers for the three enzyme systems were assigned. Assignment numbers started with the band nearest the origin (smallest Rm) and ended with band nearest the anode (largest Rm). Figure 2 shows the locations, band numbers, and Rm values for all bands observed in the *J. ashei* samples.

To eliminate the possibility of error in zymogram band interpretation from one gel to the next, an evaluation of band discreteness was performed. Samples from two or more trees were mixed in various combinations and electrophoresed. These mixed sample gels were run to evaluate all bands that had Rm values differing by 0.05 or less. By running gels with mixed samples in various combinations the discreteness of closely migrating bands for each of the three enzyme systems could be ascertained. Band discreteness was maintained for all peroxidase and esterase bands observed. When in several cases α -terpineol (alcohol) dehydrogenase zymograms of mixed samples showed convergence of bands, the original two bands were counted as one band and the data sheets corrected. This convergence of α -terpineol (alcohol) dehydrogenase bands in mixed samples accounts for the absence of the band T2, T4, T8, T10, T11, T12, T14, T18, and T19 from the data and from Figure 2 (center).

After electrophoresis of all tree samples and band discreteness evaluation, each tree was scored for the presence or absence of each possible band. All 15 populations sampled were analyzed for peroxidases, and in addition six of the 15 populations (2, 4, 10, 24, 25, & 27) were analyzed for esterases and α -terpineol (alcohol) dehydrogenases.

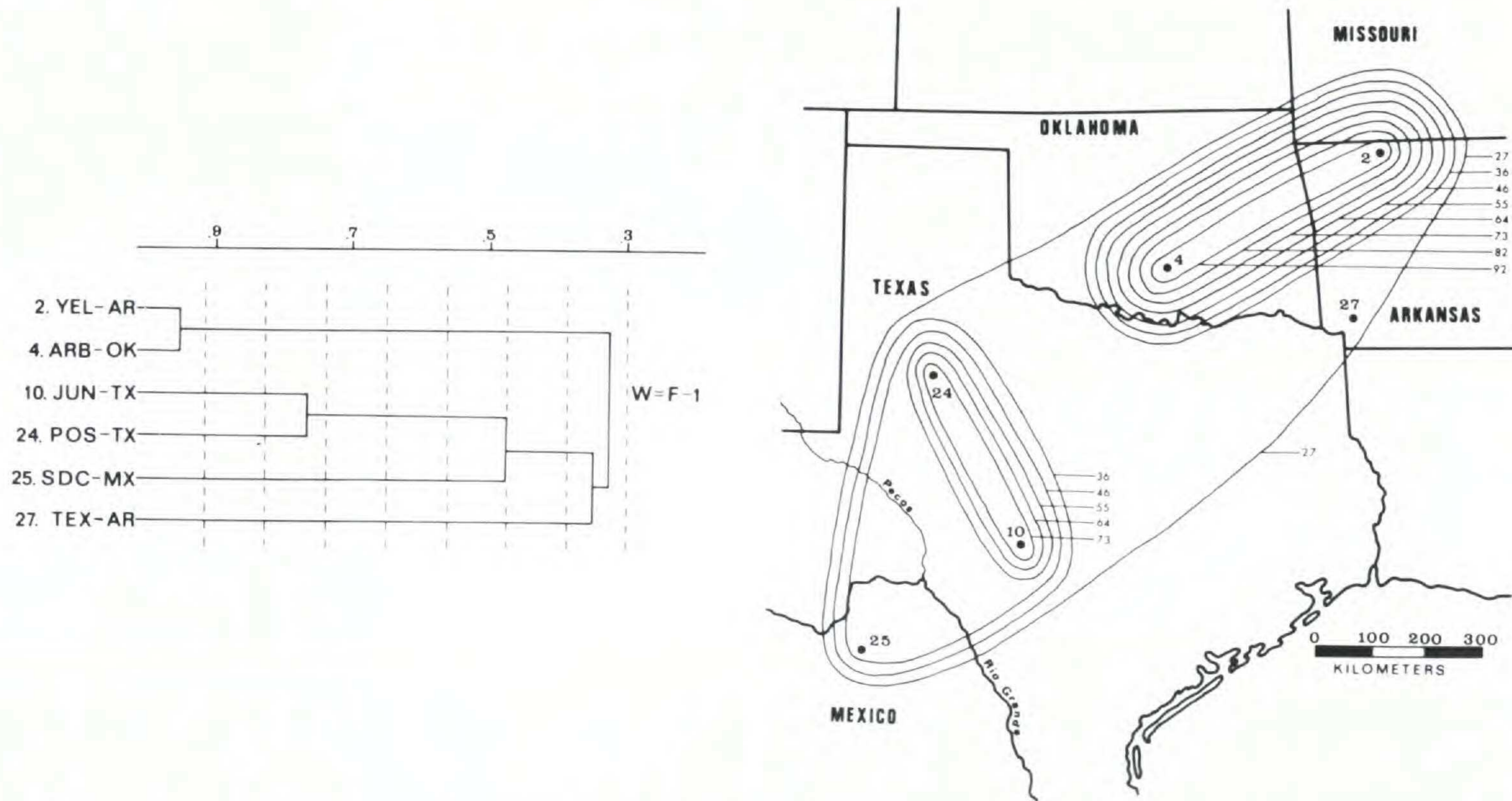


Figure 5. Phenogram and contoured similarity of six populations of *Juniperus ashei* based on 11 α -terpineol dehydrogenases, F-1 weighted. Similarity values are shown on the scale at the top of the phenogram. The dashed lines on the phenogram show the contour levels used on the contoured similarity map.

Data Analysis. As part of preliminary data analysis the average number of bands per population was calculated for peroxidases. Data were subjected to contour mapping following the methods of Adams (1970, 1974).

The raw data were also used to calculate isozyme frequencies in each population. These frequencies are the characters used in the following data analyses. The operational taxonomic units (OTU's) being evaluated are the *Juniperus ashei* populations.

Each character was analyzed to determine the importance of that character relative to the variation observed in the OTU's. Analysis of variance (ANOVA) was performed on each of the 41 band frequencies to detect by the use of the F ratio (variance among OTU's/variance within OTU's) which characters (band frequencies) exhibited statistically significant differences among the OTU's. One should note that ANOVA of qualitative characters may underestimate the F ratios, but these ratios were only used to obtain approximate weights.

A numerical taxonomic method was used to determine similarities among OTU's. The similarity measure utilized was a weighted, mean character difference (MCD) or Manhattan Metric (see Adams, 1972, for formulation).

The first analysis consisted of a combined evaluation of 31 characters (all three enzyme systems) using F-1 for the weight of each character comparison between populations. Similarity measures were also calculated for each enzyme system separately: all OTU's (15 populations) for 16 peroxidases; six OTU's for four esterases; and six OTU's for all α -terpineol (alcohol) dehydrogenases.

The single linkage cluster (SLC) method of Sneath (1957) was used for cluster analysis of the similarity matrices.

Variability within OTU's was evaluated from the raw data by using the coefficient of phenetic variation (CPV) of Gilmartin (1974) and mean similarities (\bar{S}_r). These values were determined from the combined data set of all characters (41 isozymes; three enzyme systems) and all OTU's, as well as separately for each enzyme system (15 OTU's for 23 peroxidases, six OTU's for four esterases, six OTU's for 14 α -terpineol (alcohol) dehydrogenases).

The \bar{S}_r 's and CPV's were contour mapped (Adams, 1970, 1974) to aid in the visualization of regional trends.

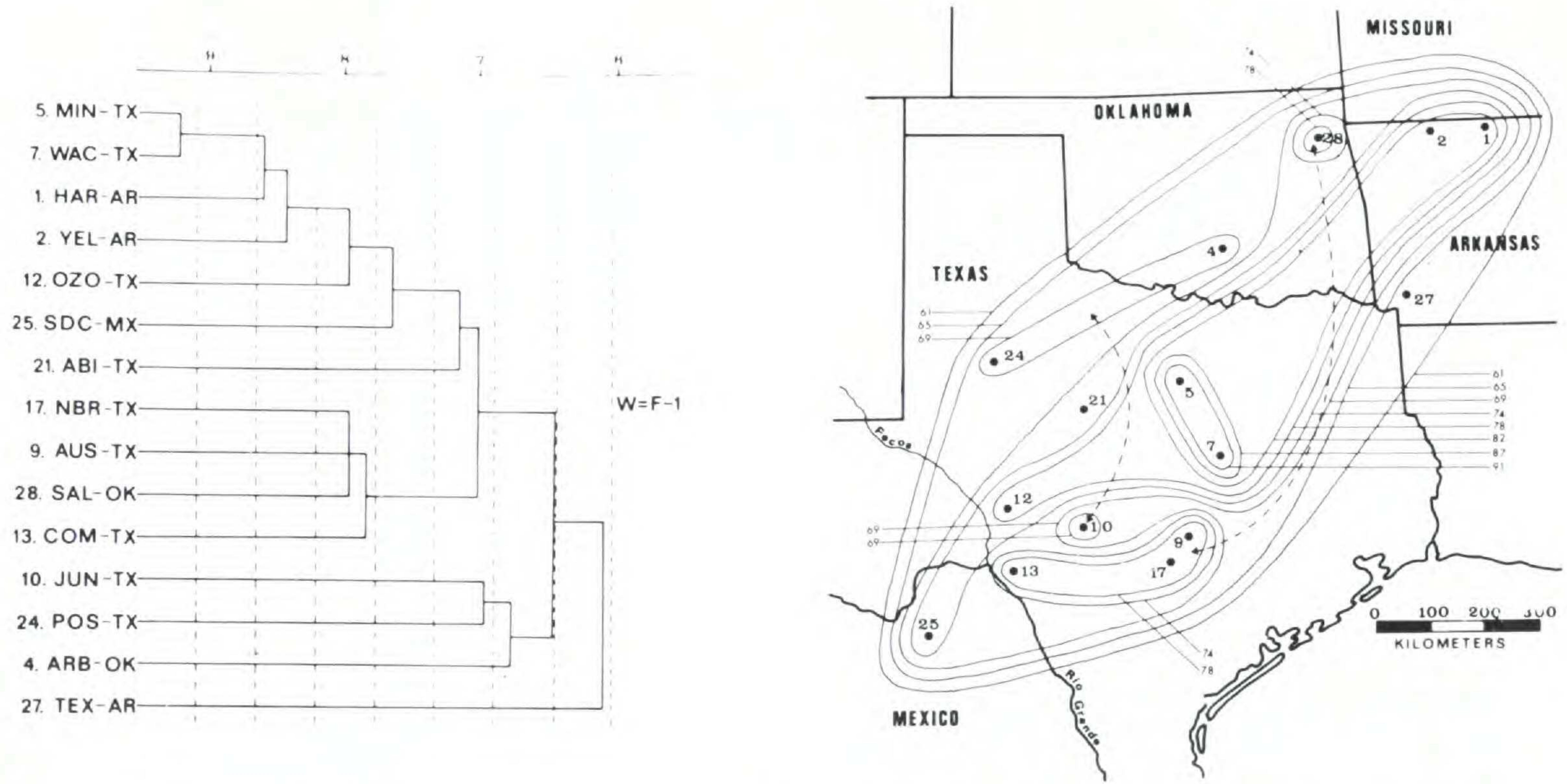


Figure 6. Phenogram and contoured similarity of 15 populations of *Juniperus ashei* based on 16 peroxidases, F-1 weighted. similarity values are shown on the scale at the top of the phenogram. The dashed lines on the phenogram show the contour levels used on the contoured similarity map.

RESULTS

Peroxidases were examined in 415 tree samples (15 populations) and esterases and α -terpineol (alcohol) dehydrogenases in 171 tree samples (six populations). An individual tree had from three to 12 peroxidase bands with an average of 7.4, four to nine α -terpineol (alcohol) dehydrogenases with an average of 5.5, one to four esterases with an average of 1.6. All *Juniperus ashei* trees examined were found to have peroxidase band 23 (P23) and α -terpineol (alcohol) dehydrogenase band 20 (T20); all other bands were variable in expression. Figure 3 shows the contour map for the average number of isoperoxidases per population. No regional trend was detected from the contour maps of the average number of peroxidases per population (Figure 3). The Hardy, Arkansas, population had the highest average number of peroxidases while the lowest average was at Texarkana, Arkansas. Further to the southwest an increase in the average number of peroxidases occurs in populations 5 (Mineral Wells, Tx.) and 7 (Waco, Tx.) on the northeast edge of the Edwards Plateau of Texas. In the central Edwards Plateau area (populations 17 to 24) there is another decrease in the average number of peroxidases. At the southwest extreme of the *J. ashei* distribution there is a slight increase in the average number of peroxidases (populations 12, 13, & 25). One might note that the Texarkana population (27), which has fewest bands, is rather small and isolated; whereas, the Junction population (10), with few bands, is large and centrally located in the *J. ashei* range.

It could be proposed that the large average number of isoperoxidases in the Hardy population (1) may be the result of hybridization between *Juniperus ashei* and *J. virginiana*, both of which occur in this area. Several lines of evidence cast doubt on this proposal. Adams (1975, 1977) and Adams and Turner (1970) could not find any evidence for hybridization between these two species using 59 terpenoids and 15 morphological characters in analyses. When sampling populations it was common to collect both species in mixed populations (populations 1, 2, 7, 9, 27, & 28). Electrophoresis was always performed on both the *J. ashei* and *J. virginiana* samples. These two species could be distinguished by their isoperoxidase zymograms and had only bands P10 and P17 in common. There is thus considerable evidence that hybridization between *J. ashei* and *J. virginiana* is not responsible for the higher number of

peroxidases in some populations where their distributions overlapped.

When ANOVA was performed on 41 characters (band frequencies), 31 of the 41 characters had significant F ratios ($P = .05$). Three bands (P3, T17, T22) had infinite F ratios; these characters had no variance within OTU's. Band P3 was found exclusively in all trees of population 1, T17 exclusively in all trees of population 25, and T22 exclusively in all trees of population 27. Two characters (P23, T20) were found in all samples and therefore had no variation in frequency among OTU's. Five characters (P6, P9, P11, P12, P21) had F ratios less than 2.30 ($F_{.05} = 1.77$, $df = 14/400$, for continuous data). It should be noted that the significance of F is underestimated when using presence or absence data and therefore this statistical test is conservative under these circumstances.

The phenograms and contour maps derived from clustering of similarity values are shown in Figures 4 (four esterase), 5 (11 α -terpineol dehydrogenases), 6 (16 peroxidases), and 7 (all 31 isozymes combined).

Contour maps derived from clustering of similarities present variable results. Each enzyme system examined gave a unique trend. Esterases (Figure 4) cluster population 4 (Arbuckle Mts.) with population 10 (Junction) as being most similar. Populations 24 (Post), 27 (Texarkana), 25 (Sierra del Carmen Mountains, Mexico), and finally population 2 (Yellville, Arkansas) then tail into the cluster (Figure 4).

α -Terpineol (alcohol) dehydrogenases (Figure 5) show a different pattern than seen with the esterases. The α -terpineol dehydrogenases divide the populations studied into two major groupings: a northern group composed of populations 2 (Yellville, Arkansas) and 4 (Arbuckle Mts.) and a southern group composed of populations 24 (Post), 10 (Junction), and 25 (Sierra del Carmen Mts., Mexico) with population 27 (Texarkana) coming in last.

Similarities based on peroxidases show a cluster pattern different from that shown by the other two enzyme systems (Figure 6). A cluster of populations occurs along a line running from population 25 (Sierra del Carmen Mts., Mexico) in the southwest to population 1 (Hardy) in the northeast. Populations 12 (Ozona, Texas), 5 (Mineral Wells, Texas), and 7 (Waco, Texas) lie on this northeast-southwest line and cluster with populations 25 and 1. On either side of this line populations show disjunctions in similarity cluster-

ing. One group is composed of populations 13 (Comstock, Texas), 17 (New Braunfels, Texas) and 9 (Austin, Texas) — three populations in the southern portion of the Edwards Plateau along with population 28 (Salina, Oklahoma). A second disjunct clustering is composed of population 10 (Junction) and populations 24 (Post) and 4 (Arbuckle Mts.). The Texarkana population (27) is the last to cluster with the other *Juniperus ashei* populations based on similarity of peroxidases.

Clustering of populations using all isozymes studied (Figure 7) is almost identical to that seen for peroxidases. The only major difference between the contour maps of similarities based on all 31 isozymes and that based on the peroxidases is that population 4 (Arbuckle Mts.), for all isozymes, clustered in with those populations associated with the northeast-southwest cluster, rather than with the population 10 (Junction) — population 24 (Post) disjunct cluster. As with the peroxidases, population 27 (Texarkana) clustered at the least similarity to the other *Juniperus ashei* populations.

Contour maps of mean similarity (\bar{S}_r) and coefficient of phenetic variation (CPV) for esterases (Figure 8) and α -terpineol (alcohol) dehydrogenases (Figure 9) are almost identical. For esterases, the highest \bar{S}_r and lowest CPV were in populations 2 (Yellville, Arkansas), 10 (Junction), 24 (Post) and 25 (Sierra del Carmen Mts., Mexico) while Texarkana (27) showed the lowest \bar{S}_r and highest CPV.

The peroxidases (Figure 10) have the lowest \bar{S}_r and highest CPV in populations 10 (Junction), 24 (Post) and 14 (Arbuckle Mts.). High \bar{S}_r and low CPV were found in populations 7 (Waco, Texas), 9 (Austin, Texas) and 1 (Hardy, Arkansas). All other populations had intermediate \bar{S}_r 's and CPV's based on peroxidases.

The combined data based on 41 isozymes (Figure 11) showed the lowest \bar{S}_r for populations 10 (Junction), 27 (Texarkana), and 4 (Arbuckle Mts.). The highest \bar{S}_r was seen in population 1 (Hardy). Figure 11 also shows the lowest CPV was in populations 24 (Post), 25 (Sierra del Carmen Mts., Mexico), 7 (Waco, Texas), 1 (Hardy, Arkansas), and 2 (Yellville, Arkansas). High CPV's are seen in populations 24 (Abilene, Texas), 12 (Ozona, Texas), 13 (Comstock, Texas), and (Texarkana), indicating that these populations are not very homogeneous.

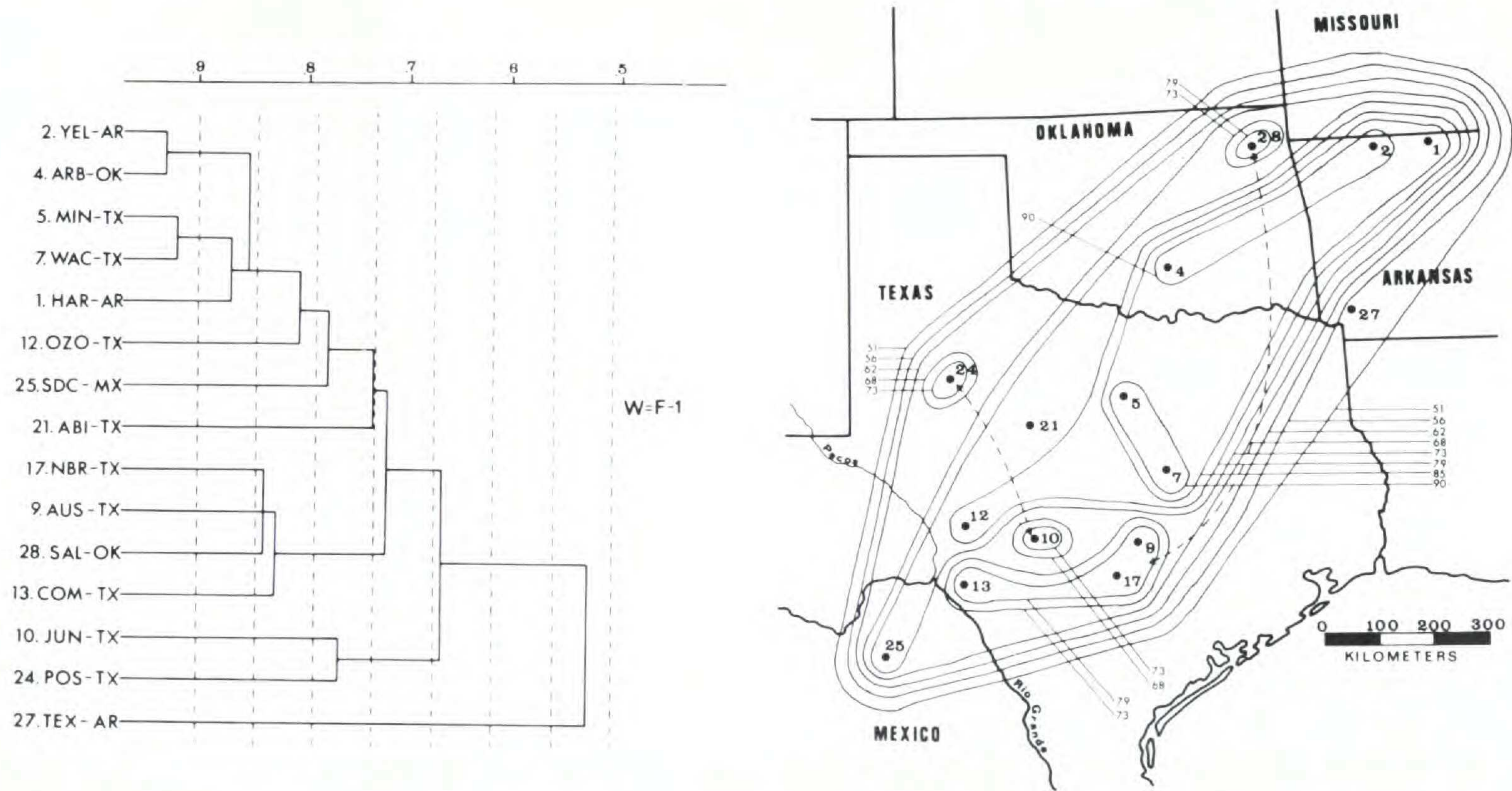


Figure 7. Phenogram and contoured similarity of 15 populations of *Juniperus ashei* based on 31 isozymes, F-1 weighted. Similarity values are shown on the scale at the top of the phenogram. The dashed lines on the phenogram show the contour levels used on the contoured similarity map.

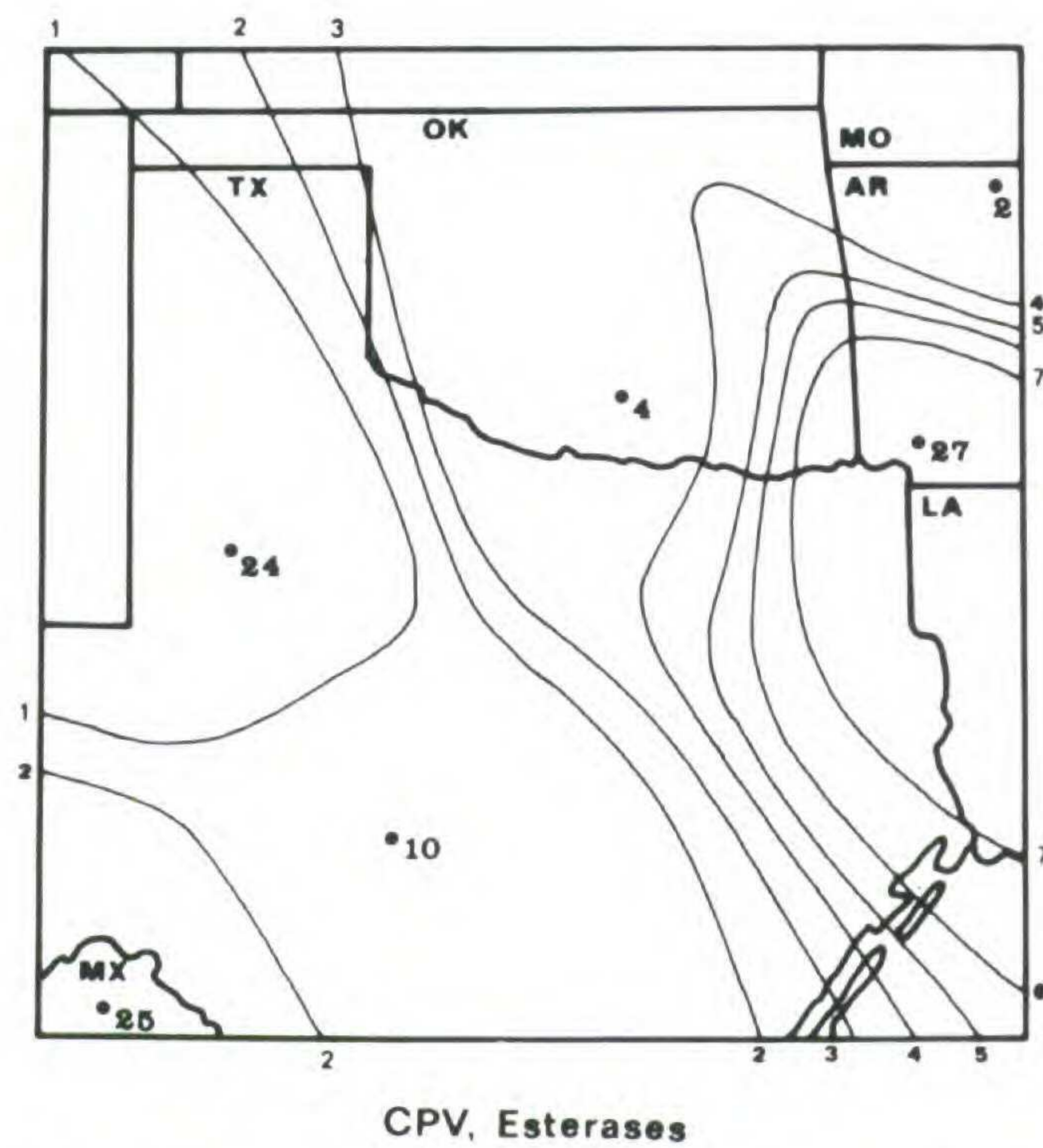
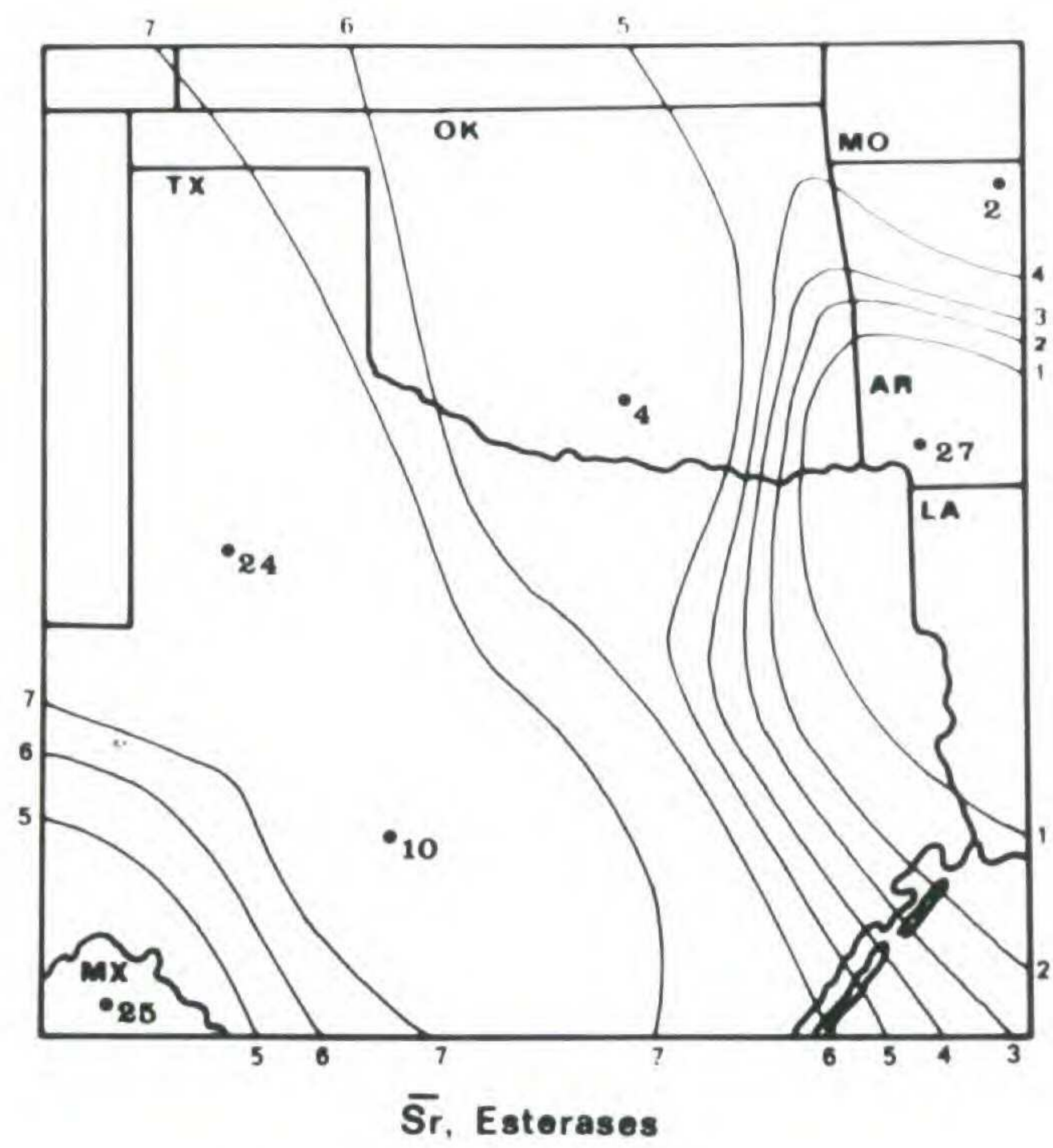


Figure 8. Mean similarity (\bar{S}_r) and coefficient of phenetic variation (CPV) contour maps for 4 esterases. \bar{S}_r , Esterases (Contours: 1 = 0.15; 7 = 0.64). CPV, Esterases (Contours: 1 = 40.4; 7 = 92.7).

CONCLUSION

Isozyme analysis in *Juniperus ashei* gives discordant results. Contour mapping of similarity values (Figures 4–7) reflects different trends for each enzyme system studied. The map of similarity values based on all enzyme systems investigated, as well as each enzyme system separately, (Figure 7) is difficult to interpret biologically. The greatest difficulty in understanding lies with the disjunct (mosaic) clustering pattern of populations on the Edwards Plateau of Texas with isolated populations in Texas and Oklahoma. There are several possible explanations for these disjunct clusterings. If selection is acting to control the isozyme patterns seen in each population then parallel (convergent) selection at the microhabitat level may be responsible for these disjunct population clusters. This idea would be compatible with results found in *Avena barbata* (Allard *et al.*, 1972a). Selection was shown to occur at the microhabitat level within distances of several feet but only one or two loci were involved.

It is also possible to explain these mosaic clusterings of populations if many of the isozymes bands are not under selection, but represent random events, responsible for the disjunct similarity of populations. Several investigators have taken this neutralist approach in explaining results similar to those found in *Juniperus ashei*. Differences found in *Cryptomeria japonica* and *Thujopsis dolabrata* could best be explained by random drift followed by geographic isolation since no apparent habitat differences could be detected (Sakai *et al.*, 1971; Sakai & Park, 1971).

These disjunct clusterings of populations of *Juniperus ashei* may have occurred as a result of a lack of homology between isozyme bands having the same relative migration (R_m) values. Although polyacrylamide gel electrophoresis is one of the best methods for separation of isozymes, it is a crude technique at the molecular level. It is highly probable that different amino acid substitutions could result in isozymes with identical overall charges and that these proteins would be of similar size. Thus, two polypeptides with different amino acid composition, and perhaps different biological functions, would migrate to the same location in a gel. An isozyme investigator would then consider these biologically different molecules as homologous since they had the same R_m values. This problem of lack of homology is inherent in electrophoretic studies where

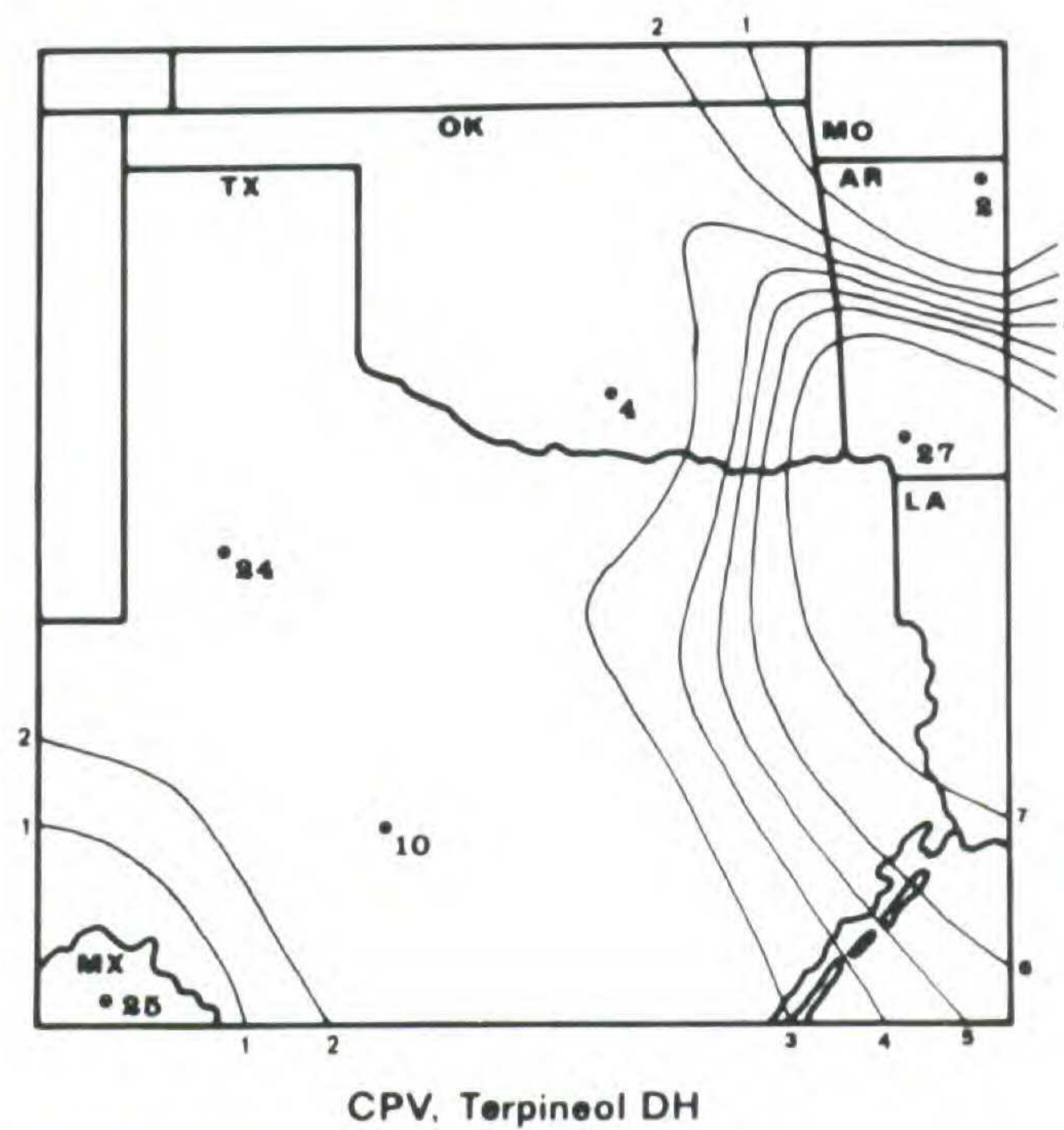
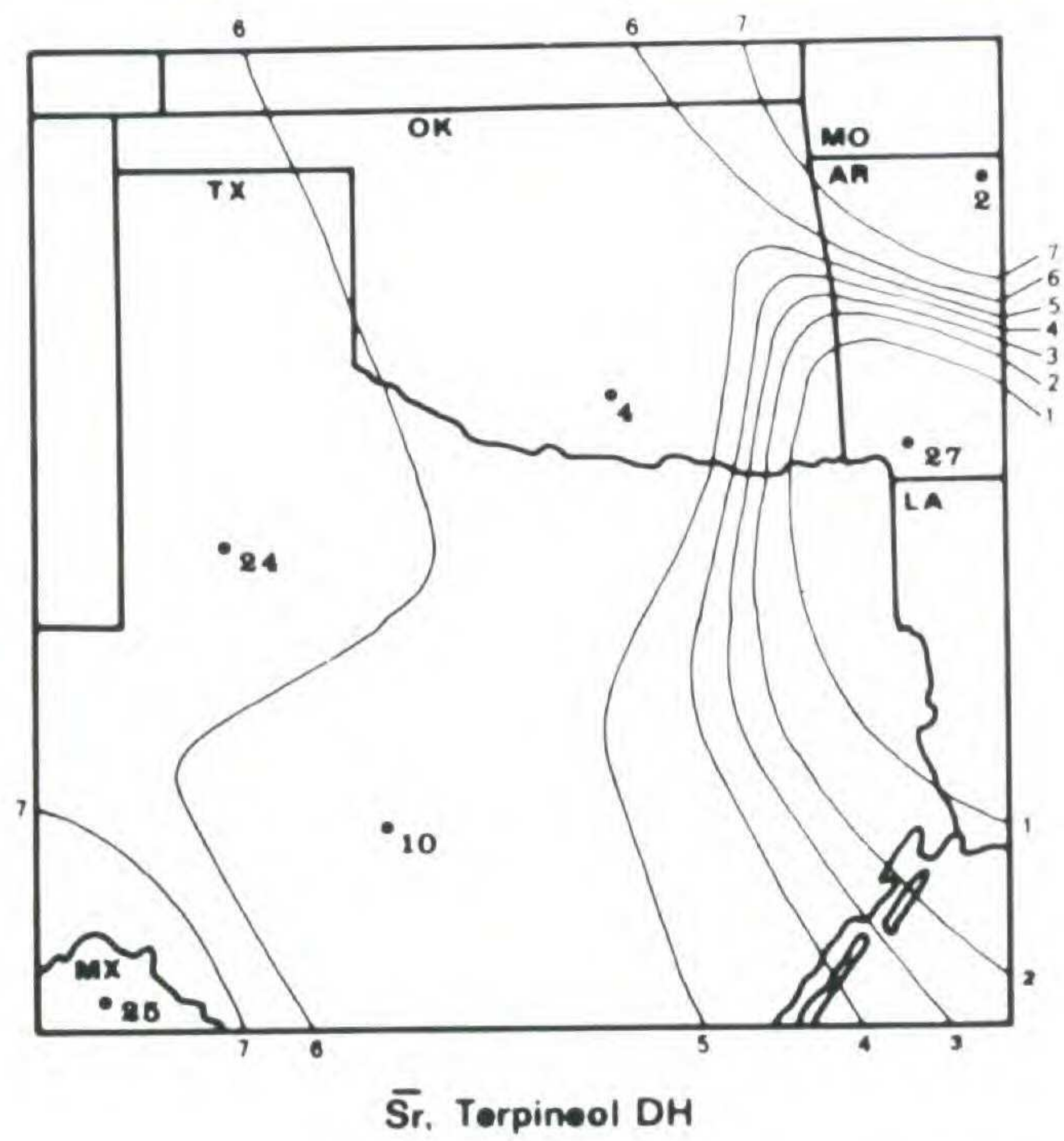


Figure 9. Mean similarity (\bar{S}_r) and coefficient of phenetic variation (CPV) contour maps for 14 α -terpineol dehydrogenases. \bar{S}_r , α -terpineol dehydrogenases (Contours: 1 = 0.79; 7 = 0.98). CPV, α -terpineol dehydrogenases (Contours: 1 = 2.34; 7 = 30.55).

band frequencies are used as the raw data for studies of genetic diversity. In isozyme investigations there is no way, short of amino acid sequencing, to know when this lack of homology is astigmatizing results, although genetic studies will help resolve some of these homologies.

Unfortunately, it appears that genetic analyses would be necessary in many populations of this taxon to resolve this problem.

It is not possible at this time to determine which of the above hypotheses best explains these unusual disjunct clusterings of populations. Perhaps no single factor but a combination of all three, to varying degrees, is responsible for the distributional patterns seen in *Juniperus ashei*. Microhabitat selection, random drift followed by geographical isolation, and lack of isozyme band homology may have combined to produce these disjunct clusterings.

These results based on contour maps of similarity in isozymes do not correspond with the patterns seen by Adams and Turner (1970) and Adams (1975, 1977), in investigations of the same populations using morphological and terpenoid data. Using terpenoid data, Adams (1975, 1977) found two major groupings (Figure 12) of *Juniperus ashei* populations; a southwestern group (populations 17, 12, 13, 25, & 26), and a northern cluster (all other populations). This lack of correspondence is comparable to other studies where isozyme, morphological and other data were compared. Gottlieb (1974b) could not divide populations of *Stephanomeria exigua* spp. *carotifera* into coastal and inland ecotypes using isozyme data as was done using morphological characteristics (Gottlieb, 1971b). Muhs (1974) could not find any trends in isozymes which correlated to coastal-interior populations of *Pseudotsuga menziesii* distinguished morphologically (Tusko, 1963).

There did not seem to be any correspondence between population size or distribution and the amount of isozyme variability detected for individual enzyme systems or combined isozyme data. Examination of small isolated populations revealed (Figure 11) a high degree of variability in population 27 (Texarkana, Arkansas) yet very little variability was found in population 24 (Post, Texas). Populations on the Edwards Plateau of Texas are in some cases highly variable (population 21, Abilene, Texas) and in others are very homogeneous (population 7, Waco, Texas). Thus, neither population size nor central versus peripheral location was correlated with the amount of variability seen in these isozymes. Similar

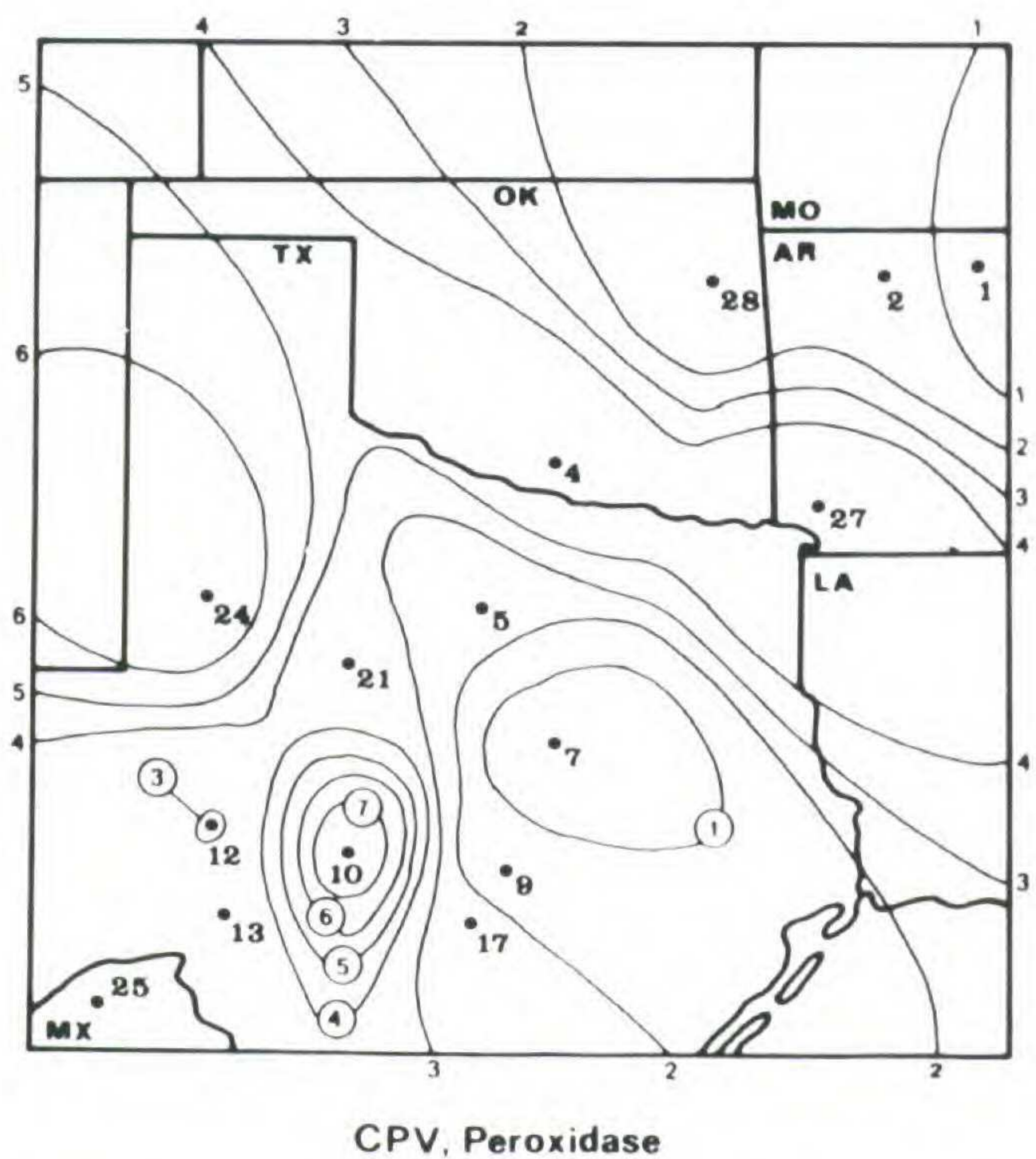
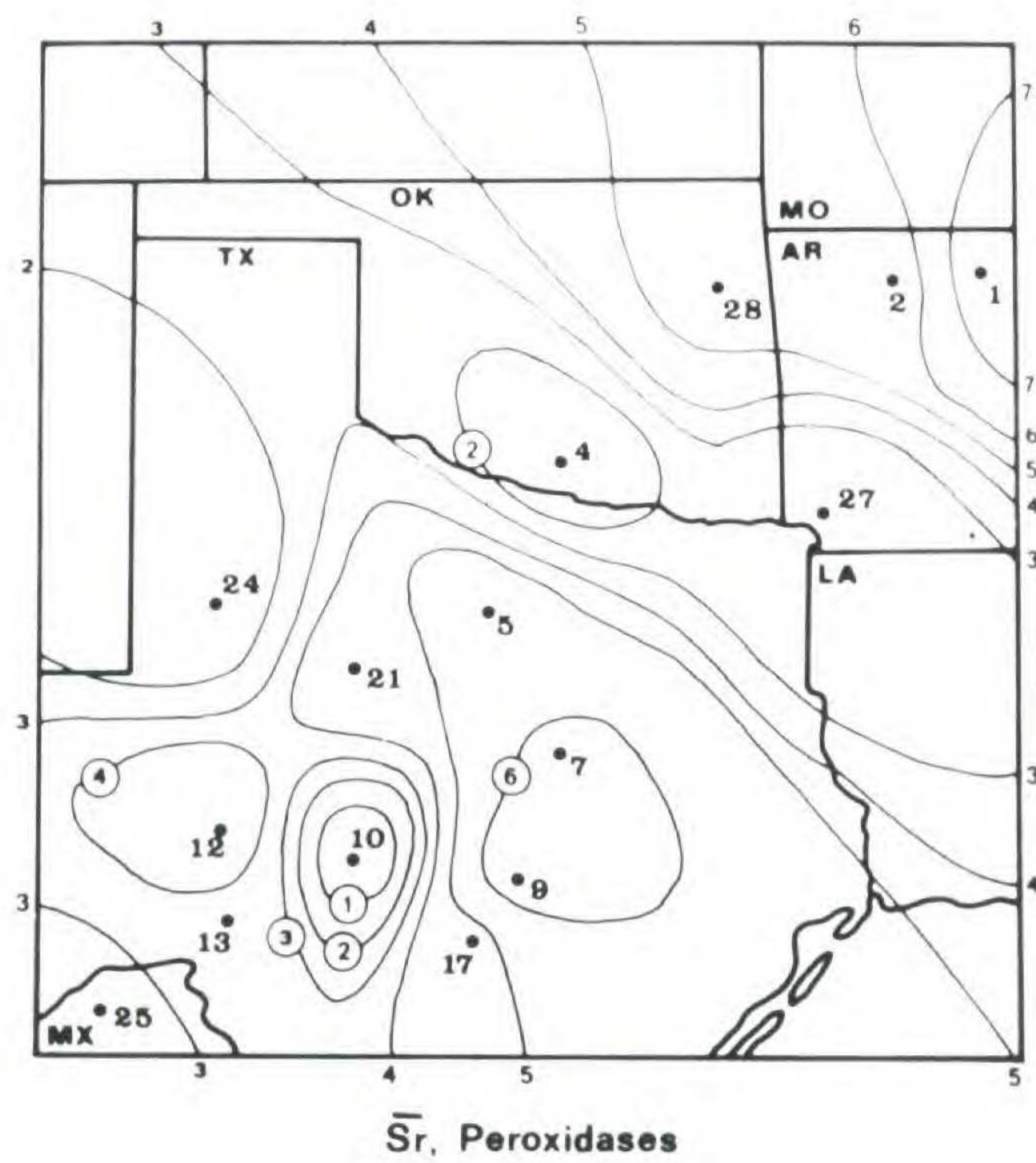


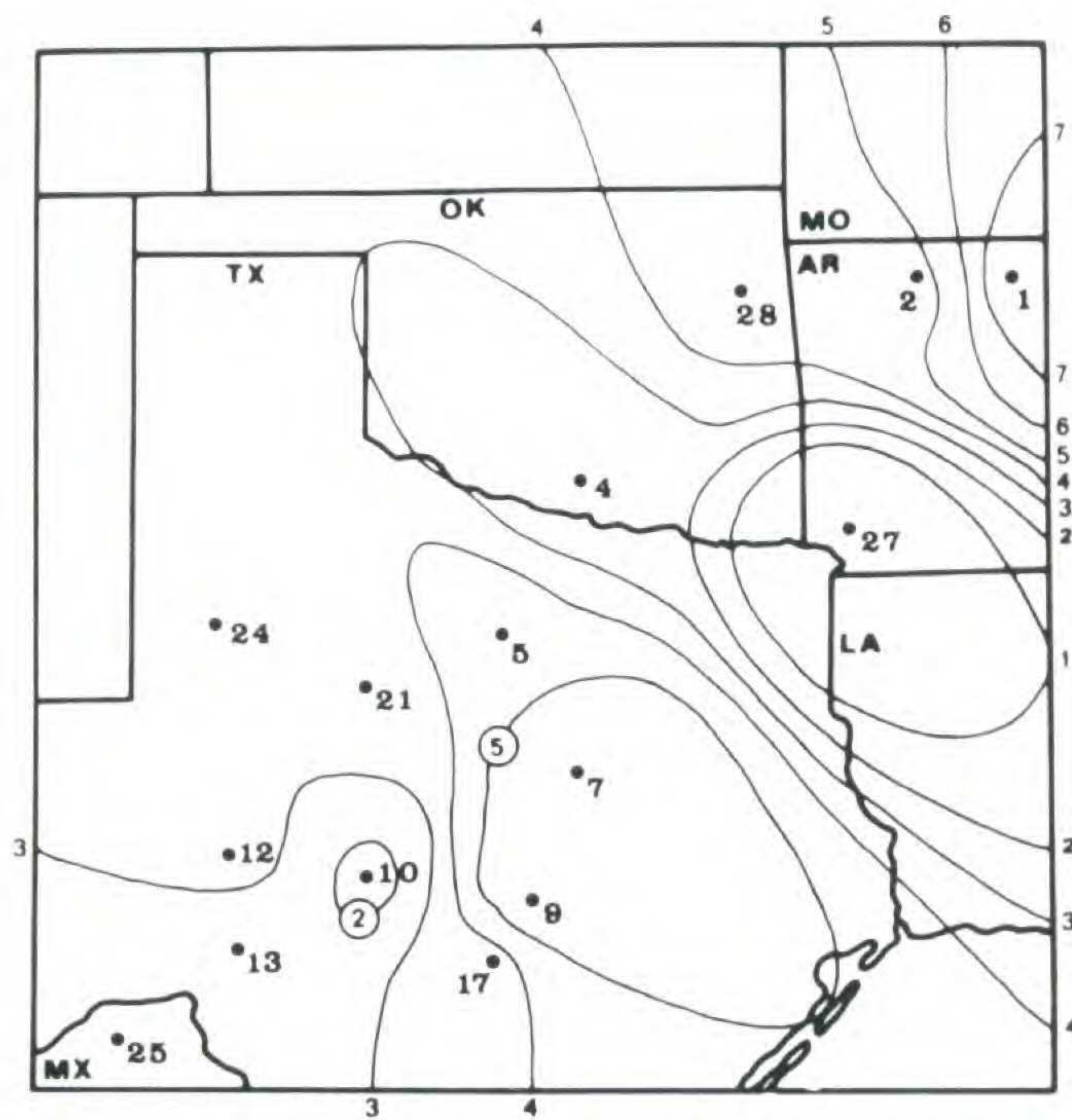
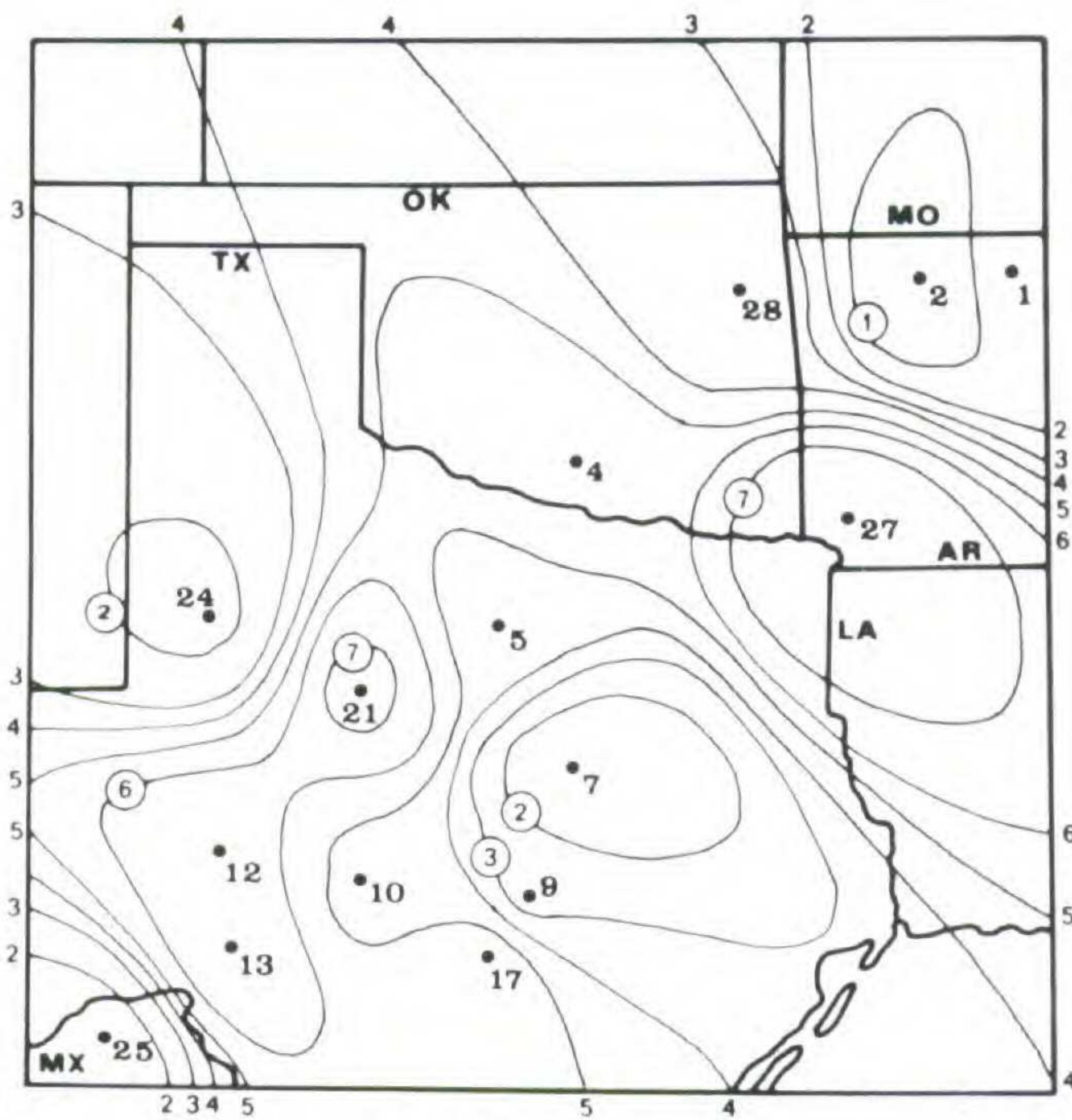
Figure 10. Mean similarity (\bar{S}_r) and coefficient of phenetic variation (CPV) contour maps for 23 peroxidases. \bar{S}_r , peroxidases (Contours: 1 = 0.54; 7 = 0.86). CPV, peroxidases (Contours: 1 = 13.45; 7 = 31.50).

results have been reported by Gottlieb (1974b) in *Stephanomeria exigua* spp. *carotifera*. It should also be noted that populations at the northern (1 & 2, Ozark Mts., Arkansas) and southern (25, Mexico) extremes of *Juniperus ashei* distribution are fairly homogeneous (see Figure 11) in most analyses.

The lack of continuity in the pattern of genetic diversity over short distances can also be seen (Figure 10) for peroxidases. Population 12 (Ozona, Texas) and population 10 (Junction, Texas) occur approximately 160 kilometers apart in the southwestern part of the Edwards Plateau in similar habitats. Population 10 is highly variable and population 12 is intermediate in variability for peroxidases. Approximately 320 kilometers northeast of population 10, the Waco, Texas, population (7) is very homogeneous for peroxidases.

These inconsistencies in *Juniperus ashei* with respect to the amount of variability associated with population size or location and the disjunct clusterings based on similarities raise questions about our concept of breeding population size, gene flow, and natural selection. If selection is the major force responsible for the isozyme patterns seen in *J. ashei*, then the population size upon which selection has acted must be relatively small, while the terpenoids and morphology seem to be under very broad regional selection with ancestral gene pools (see Adams, 1977). Very localized selection is supported by the results obtained in *Avena barbata* (Allard *et al.*, 1972a) where significantly different isozyme patterns were detected within several feet periodically over a geographically continuous distribution of this species. Selection was acting at the microhabitat level producing isozyme patterns which could be correlated with mesic or xeric habitats.

Two factors are important in the size of breeding populations of *Juniperus ashei*. These factors are pollen and seed dispersal distances (Stebbins, 1950). Which factor is of greater importance is not known, but some idea can be obtained from related information. Studies of several wind pollinated species have shown that 95% of the dispersed pollen fell within 60 feet for Douglas fir; 55 feet for pinyon; 1000 feet for elm; and 130 feet for spruce (Faegri & Van der Pijl, 1971; Percival, 1965; Wright, 1953). However, it is estimated that in a related species, most of the seed is dispersed by birds within 17 feet of its source (Phillips, 1910). Birds are of primary importance in the distribution of juniper seeds (Livingston,

 \bar{S}_r , 41 Isozymes

CPV, 41 Isozymes

Figure 11. Mean similarity (\bar{S}_r) and coefficient of phenetic variation (CPV) contour maps for 41 isozymes. \bar{S}_r , 41 isozymes (Contours: 1 = 0.64; 7 = 0.87). CPV, 41 isozymes (Contours: 1 = 11.50; 7 = 20.20).

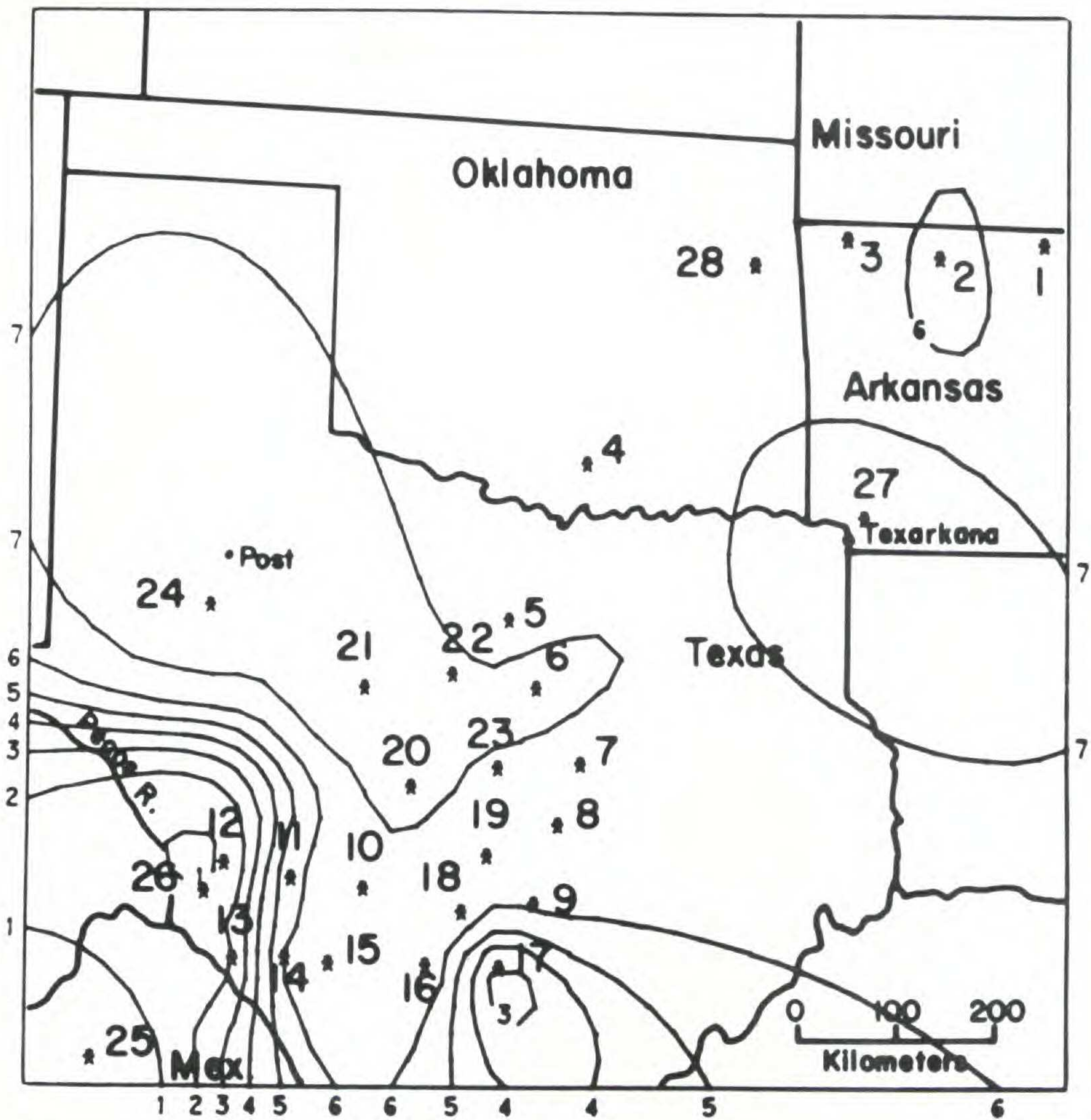


Figure 12. Contoured principal coordinate 1 of similarity measures based on 59 terpenoid characters, F-1 weighted. From Adams (1977). This same pattern was also found in 15 morphological characters, analyzed in like manner.

1972; Phillips, 1910) and arguments could be made that the disjunct similarity clusters of populations seen in Figure 7 could be explained by bird dispersal of seed. Thus, it seems highly improbable to have such large disjunct similarities, especially the southern Texas-northeast Oklahoma disjunction, with no populations showing similar isozyme patterns in between these two locations. This low probability is also supported by the report that Bohemian waxwings (*Ampelis garrulus*) have been shown to pass more than 900 berries of *J. scopulorum* in four hours (Phillips, 1910). This fact implies that long distance dispersal would be less likely. Definite

answers to questions concerning pollen and seed dispersal in *J. ashei* cannot be given here, but these estimates from other juniper species probably approximate the conditions found in this species.

The results of this isozyme investigation of *Juniperus ashei* raise as many, if not more, questions than they answer. This situation is in keeping with results of many current isozyme investigations. Since isozyme investigations in higher plants are relatively new in systematic botany it is not surprising there are many questions at the completion of preliminary studies.

Until the genetic basis of these isozymes in *Juniperus ashei* has been examined, we can only conclude that the use of isozyme banding as a taxonomic character is not feasible and certainly does not agree with the considerable mass of information supplied by the morphological and terpenoid characters previously studied in this taxon.

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